

Title: Procurement, Processing, Storage, Distribution and Study of Human Tumor Cell Cultures and Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. James T. Duff

Objectives: Part A is for the procurement, processing, distribution and study of human tumor cell cultures. Serum samples and skin punch biopsy material will be collected from the patient and will be available to recipients of the tumor material, if necessary. In addition to the characterization and distribution of these materials, research studies will be directed toward the detection of a viral genome in these cells. The human tumor material is obtained primarily from hospitals in the San Francisco Bay Area and other collaborating contractors in the Special Virus Cancer Program.

Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera cell cultures and clinical materials submitted by other SVCP contractors. Upon request, virological identification of isolates is made as to species and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: Thirty-eight human tumours have been cultivated. All viable cultures were photographed and from 3 to 11 ampoules of 6 tumours have been stored in liquid nitrogen. All tumour culture data, patient history and cell storage data is now being organized for deposition in our computer.

The compound cytocholasin B, which produces multinucleation and at high concentration causes enucleation of cells, is being studied for use in virus genome rescue experiments.

The use of antilymphocyte serum treated mice in assessing the malignancy of human tumour cells is continuing with very promising results.

Studies are proceeding with efforts to produce malignant transformation of normal human cells in vitro using chemical carcinogens and/or certain oncornaviruses.

Nine hundred and fifty-five samples were received (Feb. 1 - May 30) from SVCP laboratories to be tested for mycoplasma contamination. One hundred thirty-two have been found to be positive. This represents the largest number of mycoplasma

samples received in any 4-month period since the inception of the contract seven years ago.

Studies continue in an effort to understand the interaction of mycoplasmas with cells cultured in vitro and the effects of new mycoplasma inhibitors.

Collaborative studies were undertaken with Drs. Todaro and Aaronson on isotope labelling and density gradient separation of mycoplasma contaminants in cell cultures. All species, except M. salivarium, can be detected by this method. Quantitative aspects are now under study. Studies were completed on the sterol requirements of the T-mycoplasmas. Proof of a sterol requirement for the T-mycoplasmas and several other properties of these unusual micro-organisms suggests they be regarded as a new genus in the Class Mollicutes.

Proposed Course: (1) Continuation and expansion of collection, cultivation and characterization of human tumor cells. (2) Baking of human tumor cells in LN₂. (3) Attempts to transform normal human cells with RNA tumor viruses. (4) Continuation of studies on (a) effects of various chemical carcinogens on normal human cells in vitro, (b) quantitation of isotope labelling technique for mycoplasma detection and identification, and (c) discrimination by animals treated with antilymphocytic sera (ALS) between human normal and cancer cells, (5) Initiation of studies on cell fusion, hybridization and karyokaryon formation in vitro, and (6) detection of gs antigen in human embryonic tissue and tumor cells.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all SVCP contractors and Viral Oncology intramural staff. All of the most important viral specimens, cell cultures, sera, etc., used in the Viral Oncology Program are sent to this facility for PPLO testing and many of the SVCP contractors are dependent upon this facility for this service or as a check on their own techniques. In addition the contractor is growing human tumor cells in vitro as a resource for other SVCP contractors and for the purpose of his own research on the detection and/or isolation of a human cancer virus or oncogene.

Date Contract Initiated: June 19, 1969